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Stable Magnetic Field Gradient Levitation of *Xenopus laevis*: Toward Low-Gravity Simulation

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ABSTRACT We have levitated, for the first time, living biological specimens, embryos of the frog *Xenopus laevis*, using a large inhomogeneous magnetic field. The magnetic field/field gradient product required for levitation was 1430 kG^2/cm, consistent with the embryo's susceptibility being dominated by the diamagnetism of water and protein. We show that unlike any other earth-based technique, magnetic field gradient levitation of embryos reduces the body forces and gravity-induced stresses on them. We discuss the use of large inhomogeneous magnetic fields as a probe for gravitationally sensitive phenomena in biological specimens.

INTRODUCTION

Large inhomogeneous magnetic fields have been employed to levitate pure solids and fluids for the elaboration of their physical properties in the absence of extraneous forces exerted by gravity or the surfaces of a container (see, for example, Weilert et al., 1996). Recently, Beaugnon and Tournier established that magnetic fields produced by existing high-field solenoids can exert forces that are sufficient to levitate many diamagnetic organic materials (Beaugnon and Tournier, 1991a,b). Consequently, it should be possible to perform magnetic field gradient levitation (MFGL) of biological specimens. Such a capability would be of interest for experiments to determine the sources of gravitational sensitivity in biological systems. It would constitute an earth-based alternative to experiments done in the microgravity environment of space.

Here we present the results of an investigation of MFGL as a technique for simulating low gravity for biological systems. We have stably levitated embryos of the frog *Xenopus laevis*, using the force generated by a large inhomogeneous magnetic field. We are able to show, using measurements of the susceptibilities and densities of fractions of the embryos, that this levitation reduces the stresses on the embryos induced by gravity. In particular, levitation decreases the body forces on the embryo by an order of magnitude.

THEORY OF MAGNETIC FIELD GRADIENT LEVITATION

The idea behind MFGL is straightforward (Beaugnon and Tournier, 1991a,b; Brandt, 1989). If a diamagnetic material is placed above a strong magnet, the magnetic force exerted on it will oppose the gravitational pull of the earth. Explicitly, the force per unit mass acting on a pure material with a specific magnetic susceptibility χ_ρ (units of cm^3/g) is

$$f = \left(\frac{\chi_\rho}{2}\frac{d(B^2)}{dz} - g\right) \tag{1}$$

where g is the acceleration due to gravity, B is the applied magnetic field in gauss, and z is the height of the object in centimeters. By adjusting $d(B^2)/dz$, the effective gravity, $g_{eff} = f$, can be reduced to zero and the object levitated. Because χ_ρ is a molecular or atomic property for organic materials, every molecule or atom in the object experiences the same balance of gravitational and magnetic forces. Consequently, the value of $d(B^2)/dz$ required for levitation is independent of the mass of the object. And, more pertinent to this work, all gravity-induced internal pressure gradients or stresses in the object are absent when it is levitated. This state truly mimics weightlessness. By contrast, levitating an object by floating it in a fluid does not mimic the state of weightlessness. The forces that buoy the object act only at the object's surface and do not affect the internal pressure gradients and stresses. For diamagnetic materials, this levitation can be stable, because $\nabla^2 B^2 \leq 0$ (Brandt, 1989).

The levitated state of a heterogeneous material, such as a biological specimen, can only approximate weightlessness. Each type of molecule within a specimen has its own χ_ρ and therefore experiences its own g_{eff}. When a heterogeneous object levitates, the effective gravity or body force acting on the ith constituent of the object is

$$g_{lev,i} = g\left(1 - \frac{\chi_{\rho i}}{\langle\chi_\rho\rangle}\right) \tag{2}$$

where

$$\langle\chi_\rho\rangle = \frac{\Sigma_i m_i \chi_{\rho i}}{\Sigma_i m_i} \tag{3}$$

and where $\chi_{\rho i}$ and m_i are the specific susceptibility and mass of the ith constituent, respectively. The smaller the variation in the χ_ρ, the better MFGL simulates weightless conditions.

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MATERIALS AND METHODS

Eggs, embryos, and fractionation

Frogs (*Xenopus laevis*) were obtained from Xenopus I, and were induced to ovulate by injection of human chorionic gonadotrophin from Sigma Scientific. Before levitation, the eggs (see Fig. 1) were fertilized and dejellied according to standard protocol, and the embryos were placed in a saline solution, one-third strength MMR (33 mM NaCl, 0.67 mM KCl, 0.67 mM CaCl, 0.33 mM $MgCl_2$, 1.67 mM HEPES, pH 7.4) (Kay and Peng, 1991; Denegre and Danilchik, 1993). The fractions were obtained by centrifugation of homogenized embryos. The three well-separated fractions were 1) a pellet that consisted mostly of protein in the form of yolk platelets and pieces of membrane and cortex, 2) water-rich cytosolic supernatant material, and 3) lipids.

Susceptibility and density measurements of the embryos and fractions

The χ_ρ of the fractions and $\langle\chi_\rho\rangle$ of the whole embryos with only a small amount of saline were measured using MFGL. At a fixed vertical position z, the levitating force per unit mass is directly proportional to χ_ρ and the square of the current, because the magnetic field and its gradient are each proportional to the current. We obtained χ_ρ values relative to that of double-distilled water by measuring the current required to levitate double-distilled water at a given z and the current required to levitate the materials (fractions and the embryos) at the same z, and taking the ratio of the squares of the currents. We measured this ratio for a range of z for each material and took an average to produce the χ_ρ values. The height measurements were made by sighting the vertical position of the center of the material relative to a ruler placed within the bore, as shown schematically in Fig. 2 *a*. A Questar telescope with a close-focus barrel was used for the sighting. With a height resolution of 0.5 mm, we were able to measure χ_ρ to a fraction of a percent.

The droplets with frog eggs were placed in air, in the bore, of the Bitter solenoid at the Francis Bitter National Magnet Laboratory in a few steps. First a droplet was levitated and embryos were then added to it. Later, changes in the volume fraction of embryos in a levitated droplet were accomplished by either removing saline from the droplet with a pipette or by adding more embryos. The current in the solenoid was adjusted "on the fly" to maintain stable levitation. The levitation is stable in a region on the axis of the solenoid 8–10 cm above its centerline. Below 8 cm, the levitation is unstable to axial perturbations, and above 10 cm it is unstable to radial perturbations. The eggs were allowed to develop through first and, sometimes, third cleavage while levitated. Photographs were taken through a Questar telescope with a 35-mm camera.

The densities and relative volume fractions of the fractions were measured by standard techniques.

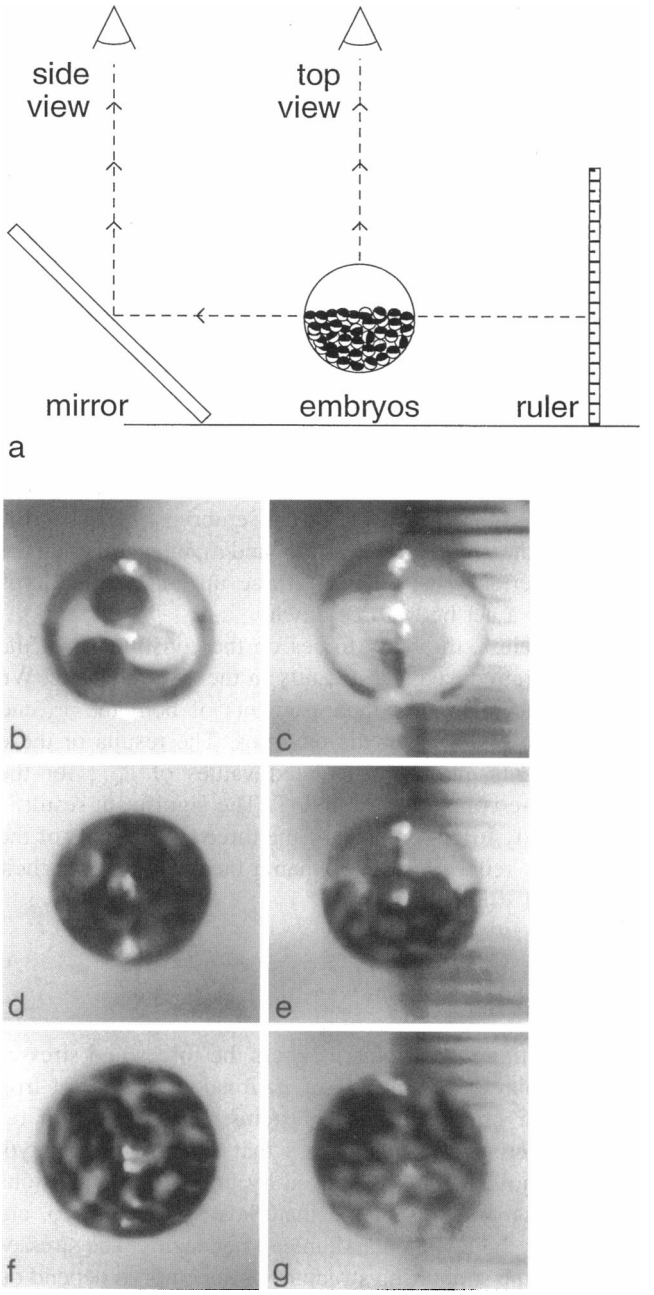

FIGURE 2 (*a*) Geometry for taking photographs of saline droplets containing fertilized eggs. A mirror was placed in the bore, in the region of stable levitation, to allow simultaneous viewing of the top and side of a sample from the top of the bore. (*b–g*) Photographs of levitated embryos. Top views (*b,d,f*) and side views (*c,e,g*) of droplets with quantities of embryos and average susceptibilities as follows: (*b,c*) three embryos, $\langle\chi\rangle = -0.72 \times 10^{-6}$ cm^3/g; (*d,e*) ~55% volume fraction of embryos, $\langle\chi\rangle = -0.70 \times 10^{-6}$ cm^3/g; (*f,g*) ~95% volume fraction of embryos $\langle\chi\rangle = -0.69 \times 10^{-}$cm^3/g. The horizontal rulings in *c*, *e*, and *g* are spaced by 1 mm. The embryos are ~1.2 mm in diameter and appear nonspherical because of the lensing effects of the droplet.

RESULTS

To demonstrate the feasibility of MFGL as a low-gravity simulation technique, we levitated living biological specimens. Frog embryos were chosen for preliminary experiments because previous investigations suggested that magnetic fields as large as 7 Tesla do not adversely affect their development (Jacobs and Fraser, 1994) and because their early development exhibits well-characterized sensitivity to gravity (Ancel and Vintemberger, 1948; Gerhart et al.,

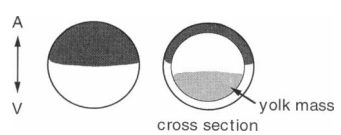

FIGURE 1 On the left is shown a sketch of a *Xenopus* embryo before first cleavage, with a cross section depicted on the right. The orientation of the AV axis is indicated at the left.

1981; Black and Gerhart, 1985; Cooke, 1986; Vincent and Gerhart, 1987; Neff et al., 1993). It should be noted, however, that frog eggs fertilized and raised in the weightless environment of space develop into normal frogs (Souza et al., 1995). In addition, the frog egg has a single axis of asymmetry, the AV axis (see Fig. 1), which is normally oriented parallel with gravity (pigmented side up). In Fig. 2 we show a schematic of the experimental setup and pictures of droplets of buffered saline solution containing different volume fractions of fertilized frog eggs stably levitated in the bore of a Bitter solenoid at the Francis Bitter National Magnet Laboratory. The droplets assume a spherical shape, indicating that gravitational stresses on them have been reduced below the strength of surface tension effects. Levitation of a pure droplet occurs at a magnetic field/field gradient product of 1370 kGauss^2/cm. The field strength in this region is $\cong$130 kGauss. The embryos reside at the bottom of the droplet (see Fig. 2, *c* and *e*), indicating that the saline experiences a net body force up and the embryos experience a net body force down.

Nevertheless, the body forces on the constituents of the embryos are reduced significantly in the levitated state. We demonstrate this using measurements of both the χ_ρ and densities of fractions of the embryos. The results of these measurements and the calculated values of $g_{\mathrm{lev,i}}$ for the fractions are compiled in Table 1. The significant result is that the body forces on each of the three constituents of the embryo are reduced by more than a factor of 10 from their unlevitated values.

DISCUSSION

This reduction in body forces alters the forces and stresses that may play a role in the gravitational sensitivity of frog embryos or other biological systems. First, levitation reduces to zero the normal forces that support the embryo. Second, buoyant forces, induced by gravity and variations in the mass densities of the materials in the embryo, are different in the levitated and unlevitated states. The stresses present in the supporting structure of the embryo depend on the apparent weight of the constituents of the embryo. Moreover, gradients in the concentration of molecules in solution depend on buoyant forces (Beaugnon and Tournier, 1991a,b; Pollard, 1965). When levitated, the apparent weight of the proteins in the embryo is reduced by the factor

$$\frac{U_{\mathrm{lev}}}{U} = \frac{\rho_{\mathrm{p}} g_{\mathrm{lev,p}} - \rho_{\mathrm{s}} g_{\mathrm{lev,s}}}{(\rho_{\mathrm{p}} - \rho_{\mathrm{s}})g} \tag{4}$$

where ρ_{p} and ρ_{s} are the densities of the pellet and supernatant material, respectively. Our data indicate that this factor is 0.7.

The reduction in the body forces and gravitational stresses achieved with MFGL on *Xenopus laevis* has not been matched by any other ground-based, low-gravity simulation technique. The most commonly employed technique, clinostat rotation (CR), involves rotation of specimens about an axis perpendicular to the direction of the gravitational force (Neff et al., 1993). This procedure only vector averages the gravity-induced forces to zero. It does not eliminate them. In fact, the centripetal acceleration of specimens in CR, when large enough, can potentially mimic gravity, and thus work counter to the intended effect. In addition, the rotation can cause movement of fluids within specimens. The resulting mass transport may interfere with investigations of convective processes in biological systems (Albrecht-Buehler, 1992).

Large inhomogeneous magnetic fields can also be used to continuously vary gravitational stresses. For example, the stresses on a *Xenopus* embryo that levitates at $z = +9$ cm relative to the solenoid center can be approximately doubled from their normal values simply by putting the embryo at $z = -9$ cm. The normal force would be twice and the buoyant force factor would be 1.3 times their unlevitated values. Or, in principle, the buoyant force, like the normal force, can also be reduced to zero. For embryos, the field-field gradient product at which U_{lev}/U goes to zero is higher than the value required for levitation. When U_{lev}/U is zero, all gravitationally induced concentration gradients and stresses have equilibrium values of zero. The normal contact force is not zero, however, and in fact, pushes down on the embryo.

An important issue that must be addressed when applying MFGL is the effects of large magnetic fields on the biological systems under study. These effects can be investigated, independent of the magnetic force effects, by placing samples at the center of the solenoid, where the magnetic field

TABLE 1 χ_ρ and densities of fractions of the embryos

Material	$-\chi_\rho \times 10^6$ (cm^3/g)	Density (g/cm^3)	Volume fraction (%)	Mass fraction (%)	($g_{\mathrm{lev,i}}/g$)
Double-distilled water	0.720*	1.00	—	—	—
Buffered saline (MMR)	0.720	1.01	—	—	—
Embryos#	0.690	1.09	96	95	<0.003
Pellet fraction	0.638	1.18	29	32	0.075
Cytosolic fraction	0.703	1.03	66	64	−0.02
Lipid fraction	0.646	0.85	5	4	0.06

*This value for the susceptibility of double-distilled water was assumed and used as a standard for the other susceptibility measurements (Ohlendorf, 1981).
#Approximately 95% volume fraction of embryos; see Fig. 2, *f* and *g*.

is maximum and the field gradient is zero. In fact, we have found that homogeneous magnetic fields as large as those we employed for levitation can cause some changes in the early development of a portion of the embryos (Denegre et al., manuscript in preparation). It remains to be shown, however, whether these alterations will have a significant impact on the efficacy of MFGL for investigating the sources of gravitational sensitivity in *Xenopus*.

SUMMARY

The decrease in gravitational stresses and the order-of-magnitude reduction in the body forces that we have achieved with MFGL on a biological specimen have not been matched by any other ground-based technique. Because the materials composing most biological systems are similar to those in frog embryos, we expect that levitation can reduce the gravitational stresses in a wide range of systems. These results suggest that MFGL can serve as a unique tool for investigating how biological systems transduce gravitational information.

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REFERENCES

Albrecht-Buehler, G. 1992. The simulation of microgravity conditions on the ground. *ASGSB Bull.* 5:3–10.

Ancel, P., and P. Vintemberger. 1948. Recherches sur le determinisme de la symetrie bilaterale dans l'oeuf des amphibiens. *Bull. Biol. Fr. Belg. Suppl.* 31:1–82.

Barone, R. P., and L. D. Caren. 1984. The immune system: effects of hypergravity and hypogravity. *Aviat. Space Environ. Med.* 55: 1063–1068.

Beaugnon, E., and R. Tournier. 1991a. Levitation of organic materials. *Nature.* 349:470.

Beaugnon, E., and R. Tournier. 1991b. Levitation of water and organic substances in high static magnetic fields. *J. Phys. III France.* 1:1423–1428.

Black, S., and J. C. Gerhart. 1985. Experimental control of the site of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Dev. Biol.* 108:310–324.

Brandt, E. H. 1989. Levitation in physics. *Science.* 243:349–355.

Cooke, J. 1986. Permanent distortion of positional system of *Xenopus* embryo by brief early perturbation in gravity. *Nature.* 319:60–63.

Denegre, J. M., and M. V. Danilchik. 1993. Deep cytoplasmic rearrangements in axis-respecified *Xenopus* embryos. *Dev. Biol.* 160:157–164.

Gerhart, J., G. Ubbels, S. Black, K. Hara, and M. Kirschner. 1981. A reinvestigation of the role of the grey crescent in axis formation in *Xenopus. Nature.* 292:511–516.

Jacobs, R. E., and S. E. Fraser. 1994. Magnetic resonance microscopy of embryonic cell lineages and movements. *Science.* 263:681–684.

Kay, B., and H. G. Peng. 1991. Methods in Cell Biology, Vol. 36, *Xenopus laevis*: Practical Uses in Cell and Molecular Biology. Academic Press, San Diego.

Neff, A. W., H. Yokota, H.-M. Chung, M. Wakahara, and G. M. Malacinski. 1993. Brief notes: early amphibian (Anuran) morphogenesis is sensitive to novel gravitational fields. *Dev. Biol.* 155:270–274.

Ohlendorf, D. H. 1981. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.

Pollard, E. C. 1965. Theoretical studies on living systems in the absence of mechanical stress. *J. Theor. Biol.* 8:113–123.

Souza, K. A., S. D. Black, and R. J. Wassersug. 1995. Amphibian development in the virtual absence of gravity. *Proc. Natl. Acad. Sci. USA.* 92:1975–1978.

Vincent, J. P., and J. C. Gerhart. 1987. Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev. Biol.* 123: 526–539.

Weilert, M. A., D. L. Whitaker, H. J. Maris, and G. M. Seidel. 1996. Magnetic levitation and noncoalescence of liquid helium. *Phys. Rev. Lett.* 77:4840–4843.